Optical Detection of Thrombus Formation within a Microfluidic Device using a Helium-Neon Laser as a Radiation Source

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Abstract:

Understanding the dynamics of blood clotting, or thrombosis, is critically important to clinical evaluations of patients and to research laboratories studying diseases and drug effects. Particularly, the necessary conditions for late-stage acute thrombus formation are not well understood, and instrumentation has been limited in this context. The emerging field of microfluidics has led to significant advances in examining thrombus formation *in vitro*, allowing biologically relevant geometries and the measurement of volumetric growth rates in real time. However, current techniques (antibody binding, microscopic imaging) produce data with poor temporal and spatial resolution for accurate rate measurements. We present a method for detecting thrombus formation within a microfluidic device using a helium-neon laser, taking advantage of the low optical absorbance of platelets relative to erythrocytes. This method provides sub-second time resolution and a smaller instrument footprint compared to existing art. Our method is also capable of measuring the hematocrit of the blood being tested, as established in previous art [1], and which has been shown to affect thrombus growth in the past [2].

Introduction:

The optical properties of blood have been well defined in existing literature. The most pertinent of these in our efforts is the transmissive properties of erythrocytes and platelets within the visible spectrum, since these are the primary constituents of thrombi. In the red portion of the spectrum, erythrocytes exhibit minimal transmittance, while platelets exhibit significant transmittance [3]. Under high blood shear conditions, which are analogous to those present within mammalian hearts, thrombus forms primarily composed of platelets. Thus as the thrombus grows, its transmittance increases measurably in relation to the surrounding blood.

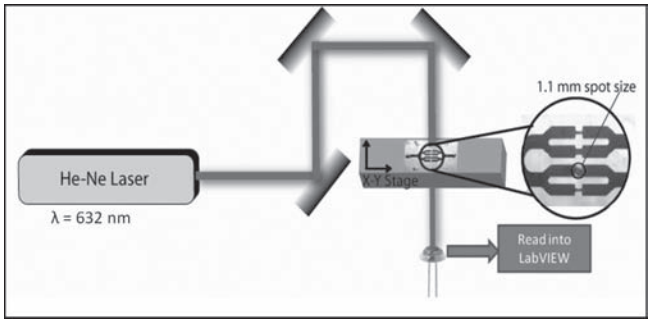

Figure 1: Schematic of the instrumentation for the single channel thrombosis measurement.

Construction:

Our research group had previously designed and manufactured a low-volume, high-throughput microfluidic device with several stenoses that exhibited shear rates between $4000s^{-1}$ and $7500s^{-1}$ (modeled through Poisuelle Flow and Particle Image Velocimetry). Porcine blood of known hematocrit (determined through centrifugation) was delivered via gravity pump into the microfluidic device which was placed upon an x-y stage for positioning.

Light from a 0.7 mW 632.8 nm helium-neon laser was passed through a series of mirrors and illuminated a single stenosis as shown in Figure 1. The transmitted light was measured by a photodiode and accompanying circuit, which provided a variable gain between 3E+5 and 1E+7. The output was read by a National Instruments Data Acquisition Device into LabVIEW where a butterworth bandpass filter was applied and a moving average was taken.

However, the aforementioned construction was only able to measure a transmittance function within a single channel. Desiring to measure thrombus formation within multiple stenoses simultaneously, our construction was modified accordingly. To accomplish this, the incident light was diverged in one dimension utilizing a BK7 uncoated cylindrical lens. This light was then passed through an acrylic aperture plate placed on top of the microfluidic device, which contained four 1000 μm apertures separated to match the channel spacing. The transmitted light was

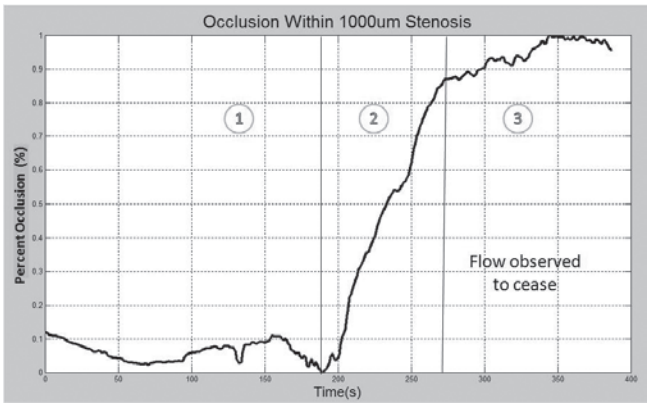

Figure 2: Photodiode output with three stage of thrombus formation labeled.

measured by a line CCD camera and read into LabVIEW for post-processing, as shown in Figure 2.

Results:

Thrombus growth was successfully measured in individual channels of the microfluidic device and was verified by comparison with microscopic imaging methods using identical blood samples and measuring at equivalent shear rates, as shown in Figure 3. Our method was able to measure the three distinct phases of thrombus formation: adhesion, acute growth, and occlusion, as shown in Figure 4. Volumetric thrombus growth could be measured with a sampling rate of up to 10 kHz, compared to microscopic imaging which operates at sub-hertz acquisition speeds.

Our method also provided a method of determining the hematocrit of the blood being measured. The initial intensity of the transmitted light is proportional to the concentration of erythrocytes, and thus can be related to the blood hematocrit with an error of approximately 2%.

Utilizing the high-throughput construction, growth has been observed in four stenoses simultaneously in addition to spatial localization of the growth within each stenosis.

Discussion:

Utilizing this method, differential thrombus formation has been observed under various blood parameters, such as

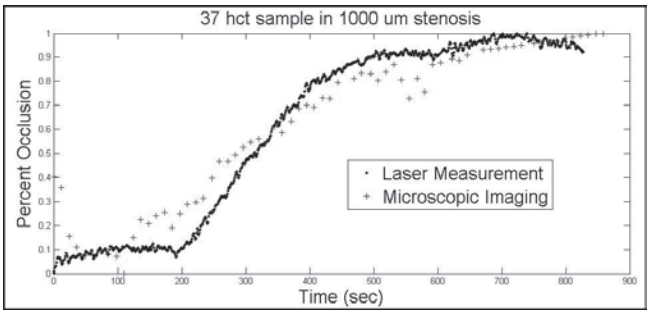

Figure 3: Comparison of laser detection with existing microscopic imaging (high frequency data corresponds to laser output).

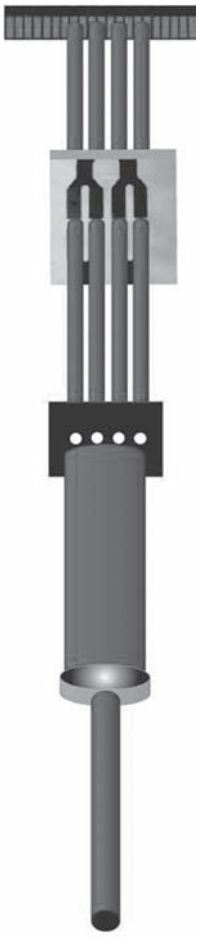

Figure 4: Schematic of the instrumentation for multi-channel thrombosis measurement.

varying hematocrit, shear rate, and dosage of acetylsalicylic acid, an anti-thrombotic drug. However, more data is required before definitive analysis can be performed from a biological viewpoint.

The methods described above provide a superior method of detection over current art that can be expanded to perform large numbers of trials simultaneously. Additional tests are being conducted to validate the accuracy of the high-throughput instrumentation in comparison to the single-channel measurements. However, initial data indicates that it will be as accurate, in addition to providing previously unavailable spatial resolution.

Conclusion:

Described is a method of quantitatively analyzing acute volumetric thrombus growth. The method described provides not only a high time resolution, but also a smaller laboratory footprint and data load than existing art. The presented method is multifunctional, allowing an assessment of acute thrombus growth rate, time to occlusion, and the hematocrit of the blood being analyzed. Additional functionality can be added such as measuring hemoglobin concentration by observing the blood's optical scattering. Utilizing the presented method, the effects of drugs such as acetylsalicylic acid can be examined under different conditions, such as varied shear rates that simulate individuals with various stages of heart disease.

Acknowledgements:

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References:

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[2] Ross, J. M., L. V. Mcintire, et al. (1995). "Platelet-Adhesion and Aggregation on Human Type-Vi Collagen Surfaces under Physiological Flow Conditions." Blood 85(7): 1826-1835.

[3] Optical Properties of Circulating Human Blood in the Wavelength Range 400-2500 nm; Andre Roggan, Moritz Friebel, Klaus Dorschel, Andreas Hahn, and Gerhard Muller, J. Biomed. Opt. 4, 36 (1999), DOI:10.1117/1.429919.